



Probing interactions within anthrax toxin by electron paramagnetic resonance



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The protective antigen (PA) moiety of anthrax toxin forms oligomeric pores that translocate the enzymatic moieties of the toxin — lethal factor (LF) and edema factor (EF) — across the endosomal membrane of mammalian cells (Fig. 1A). Characterizing interactions between membrane-inserted PA pore and LF has been challenging. No crystal structure of the pore exists, and the resolution of electron micrograph images remains low. Site-directed spin labeling EPR (SDSL-EPR) represents an attractive approach to studying PA pore-LF interactions, as it allows molecular-level resolution of interactions and can be performed with liposome-inserted forms.

Here we describe SDSL-EPR studies aimed at defining interactions of LF with the PA pore. Our results reveal a direct interaction between the extreme N terminus of LF (residues 2-5) and the Φ clamp, a structure within the lumen of the pore that catalyzes translocation. Also, we show that, upon binding of the translocation substrate to PA, LF helix α_1 pulls away from helices α_2 and α_3 and binds in the α clamp of PA. Our findings elucidate the state from which translocation of LF and EF proceeds through the PA pore. Moreover, our results exemplify the power of SDSL-EPR as for defining interactions between oligomeric membrane-spanning proteins.

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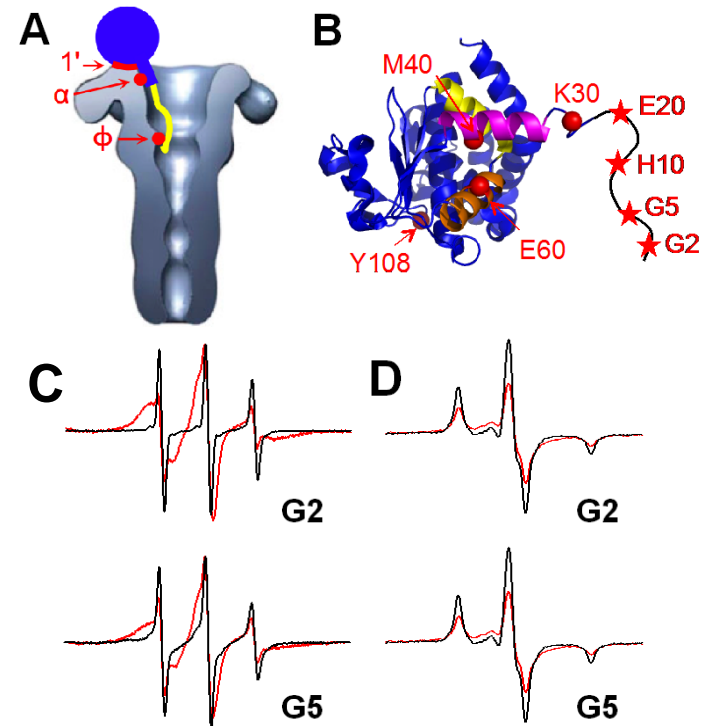


Figure 1. LF_N-PA interactions. An EM reconstruction of the PA pore with a cartoon representation of LF_N (in blue) bound (A). Crystal structure of LF_N with spin-labeled residues marked in red (B). Interaction between PA pore and LF_N residues G2 and G5 by EPR mobility (C) and distance (D) measurements. LF_N alone (Black); with PA (Red).